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Award Number: W81XWH-08-1-0301

TITLE: Aerobic Exercise, Estrogens, and Breast Cancer Risk

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REPORT DATE: November 2011

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-11-2011	Annual Summary	23 APR 2008-22 OCT 2011

4. TITLE AND SUBTITLE

Aerobic Exercise, Estrogens, and Breast Cancer Risk

5a. CONTRACT NUMBER

5b. GRANT NUMBER

W81XWH-08-1-0301

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The University of Minnesota
Minneapolis, MN 55455

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

With approximately 180,000 new cases reported in 2008, breast cancer continues to be the most frequently diagnosed non-skin cancer and second leading cause of cancer death among American women.1 A large body of epidemiological evidence suggests increasing levels of physical activity are associated with significant reductions in breast cancer risk.2, 3 The goal of this training grant was to investigate the effects of a 16-week, aerobic exercise intervention on endogenous sex hormone levels, menstrual cycle characteristics, and estrogen metabolism in sedentary, eumenorrheic, healthy premenopausal women. Main results from this exercise intervention include: 1) significant decreases in body fat and increases in lean body mass without body weight changes, 2) no significant changes in serum estradiol, estrone sulfate, testosterone, progesterone, and sex hormone binding globulin, 3) no significant changes in menstrual cycle length, and 4) limited changes in estrogen metabolism. The resulting increases in urinary 2-hydroxyestrone levels and 2-to-16-hydroxyestrone ratio are consistent with decreased breast cancer risk. These result support the hypothesis that hormonal changes, such as changes in estrogen metabolism, may be in part responsible for the favorable effects of exercise on breast cancer risk.

15. SUBJECT TERMS

Aerobic exercise, Estrogens, Sex hormones, Estrogen metabolites, Breast cancer risk, Premenopausal women

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	27	19b. TELEPHONE NUMBER *(include area code)*

INTRODUCTION

A growing body of epidemiological evidence suggests increasing levels of physical activity are associated with significant reductions in breast cancer risk. [3] The physiological mechanisms by which physical activity may help mediate breast cancer risk, however, are not well understood. The goal of

this training grant was to investigate the effects of a 16-week, aerobic exercise intervention on endogenous sex hormone levels, menstrual cycle characteristics, and estrogen metabolism of young, sedentary, healthy women. A comprehensive evaluation on women's hormonal profile included changes in circulating serum levels of estradiol, estrone sulfate, testosterone, progesterone, and sex hormone binding globulin (SHBG), changes in menstrual cycle length, and changes in urinary levels of estrone, estradiol, and their respective metabolites.

BODY

The PI has accomplished all of the expected research and training tasks outlined in the approved Statement of Work during the grant-training period 04/23/08 to 11/22/11.

First and foremost, the PI helped recruit, supervise, and manage the Women In Steady Exercise Research (WISER) study such that successful completion of 319 women (99.7% of the expected final sample size) was attained in October 2009 (Task 1). This process was a laborious one since more than 100, 000 women were contacted via email to participate, 1684 were screened via telephone, 966 were given a 2-hour orientation, and 391 were randomized into the study (212 allocated to the exercise group and 179 allocated to the control group).

In terms of breast cancer training, the PI was able to obtain invaluable clinical mentoring from Dr. Dourglas Yee, MD, at the University Cancer Center by attending weekly sessions of case discussion with him and other oncologists, surgeons, and other clinicians (Task 2). In addition, the PI has been able to further enhance her training by attending and participating in the Transdiciplinary Research in Energetics and Cancer (TREC) meeting (2009 and 2010), the American College of Sports Medicine (ACSM) (2009) meeting, the International Society for Behavioral Nutrition and Physical Activity (ISBNPA) meeting (2009), and the Era of Hope meeting (2011).

One of the most important research tasks the PI was able to accomplish during the training period was to analyze all of the serum and urine samples generated by the WISER study (Task 3). At the end of the study, the PI had analyzed approximately 7600 biological samples: 5 serum sex hormones and 12 urinary estrogen metabolites at baseline and follow up for each of the 319 women who completed the study. All serum samples were assayed in triplicate and urine samples assayed in duplicate by radioimmunoassay and high performance liquid chromatography methods, respectively.

Finally, the most challenging and time-consuming task of this grant (Task 4) has required the PI to analyze the study results, prepare manuscripts for publication, and present the results of this research at scientific meetings. With the help of the study statistician and the PI's academic mentor, the PI has completed the analysis and interpretation of all the data this October 2011. Dissemination of selected study results has been possible through oral and poster presentations. Specifically, the PI was invited to give a 20 minute presentation at the 2009 ACSM meeting in Seattle, WA and presented a poster at the 2009 ISBNPA and 2011EOH meetings in Caiscais, Portugal and Orlando, FL, respectively. Furthermore, manuscripts reporting on study results have been submitted and accepted for publication in scholarly, peer-reviewed, scientific journals. Specifically, results on the effects of exercise on endogenous sex hormones and menstrual cycle length have been published recently (July 2011) by the Cancer Epidemiology, Biomarkers, and Prevention journal. A copy of this article has been included in the appendix section of this report. A second manuscript reporting on the effects of exercise on estrogen metabolism is currently underway and expected to be ready for submission to a scholarly journal by the end of 2011. Once this

manuscript is accepted for publication, the PI will be able to finish her PhD dissertation, submit it to her academic panel and defend it to complete all requirements necessary to achieve the doctorate degree in Nutrition at the University of Minnesota. The expected graduation date is May 2012.

KEY RESERCH ACCOMPLISHMENTS

- Successful study completion by 319 women representing 99.7% of target sample size.
- Assay of all blood and urine samples (~7600 biological samples).
- Analysis and interpretation of sex hormone and urinary estrogen metabolite data.

REPORTABLE OUTCOMES

- Abstract and oral presentation given at the 2009 ACSM meeting in Seattle, WA.
- Abstract and poster presentation given at the 2009 ISBNPA meeting in Caiscais, Portugal.
- Abstract and poster presentation given at the 2011 Era of Hope meeting in Orlando, FL.
- Manuscript reporting on the effects of exercise on serum sex hormones and menstrual cycle length published by CEBP journal (**Cancer Epidemiol Biomarkers Prev 2011;20:1098-1106).**

CONCLUSION

Given that premenopausal women have been largely underrepresented in breast cancer research and that physiological changes leading to the initiation and development of breast cancer are likely to occur over a long period of time, it is of outmost importance to investigate environmental exposures and social behaviors in premenopausal women. The WISER study was a randomized clinical study investigating the hormonal effects of a 16-week, aerobic exercise intervention in young, sedentary, healthy, premenopausal women. To date, this is the largest randomized exercise human study measuring changes in endogenous sex hormone levels, menstrual cycle characteristics, and estrogen metabolism in young women.

Results from this study are extremely important in determining whether hormonal changes are viable mechanisms by which exercise helps reduce breast cancer risk. If this is the case, campaigns advocating increased physical activity may prove invaluable in terms of cancer prevention efforts. If not, results from this study would provide solid evidence, given the strength of the study design and large sample size, that this hypothesis is no longer worthwhile pursuing and suggest cancer research efforts should be allocated to other endeavors.
The results of the WISER study suggest 16 weeks of steady, moderate-to-vigorous aerobic exercise leads to significant improvements in body composition measures (such as increased muscle mass and decreased body fat without concomitant body weight changes) but no significant changes in premenopausal levels of endogenous sex hormone and SHBG or menstrual cycle length. Similarly, our research suggests aerobic exercise, at least of the

duration, length and intensity prescribed in this study, does not lead to significant changes in menstrual cycle length. Therefore, we conclude that any favorable impact aerobic exercise may have on breast cancer risk is unlikely due to changes in levels of sex hormones, SHBG or menstrual cycle length. Importantly, however, our study did find that the study exercise intervention lead to significant changes in estrogen metabolism that are consistent with decreased breast cancer risk. Culture and animal studies have shown 2-OHE_1 and 16-OHE_1 to have anti- and pro-estrogenic properties, respectively. The favorable changes found in this study include significant increases in 2-hydroxyestrone (2-OHE_1), no significant changes in 16-hydroxyestrone (16-OHE_1), and a significantly higher 2-to-16-hydroxyestrone ratio. Based on the collective results of this study, we conclude one of the possible hormonal mechanisms by which exercise may decrease breast cancer risk is through estrogen metabolism changes and not necessarily through changes in blood hormone levels or menstrual cycle characteristics.

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APPENDIX 1

Abstract for oral presentation at the American College of Sports Medicine annual conference, May 27-30, 2009 in Seattle, WA.

Title: Aerobic Exercise, Estrogens and Breast Cancer Risk in Premenopausal Women

Alma Smith[1], Andrea Arikawa[1], Katie Schmitz[2], Mindy Kurzer[1]. [1]*University of Minnesota, Twin Cities, MN.* [2] *University of Pennsylvania, Philadelphia, PA.*

PURPOSE: Currently known risk factors for breast cancer are difficult to control and modify and therefore, there is a need to identify alternative risk factors that are more suitable for intervention, especially among young women. The WISER study, Women in Steady Exercise Research, and the pilot study that preceded it investigate the effect of aerobic exercise on sex hormone levels in healthy premenopausal women.

METHODS: The WISER study is a randomized, exercise-controlled clinical trial that is randomizing 320 sedentary, healthy (BMI 18-40kg/m^2), eumenorrheic,

premenopausal women aged 18 to 30 years old into an exercise or no-exercise control group. Intervention subjects exercise aerobically for 30 minutes, 5 times a week during 4 menstrual cycles in which the workout intensity increases every four weeks by 5% of their age-predicted maximal heart rate until it reaches 80%-85%. The effect of aerobic exercise on blood sex hormones, urinary estrogen metabolites, menstrual cycle characteristics, ovulation status, measures of fitness (time to 85% max heart rate, MET-h/week), and body composition are being measured at baseline and follow-up. The study design for the completed pilot study in 15 women was identical, except for the lack of a comparison group.

RESULTS: In the WISER pilot study, after 15 weeks of aerobic exercise, there were significant reductions in body weight (-1.7 %, $p < 0.007$), BMI (-2.4%, $p < 0.01$), and fat mass (-6.5%, $p<0.04$). In contrast, a significant 10.6% increase in submaximal fitness ($p< 0.004$) was attained through the intervention. No significant changes in endogenous estrogens were observed, though the pattern of changes was consistent with a reduction of cancer risk. Levels of estrone, 16-α-hydroxyestrone, estriol, 4-hydroxyestrogens, and the ratio of 2-to-4 hydroxyestrogens were reduced while that of 2-hydroxyestrogens and the ratio of 2-to-16-α-hydroxyestrone increased.

CONCLUSIONS: Results from the pilot study showed that aerobic exercise training at the dose currently recommended for health promotion and disease prevention results in significant reductions in body fat in young eumenorrheic women. Changes in estrogens were in the expected direction, though not significant. The larger, ongoing WISER trial was designed to further investigate the effects of aerobic exercise training on endogenous sex hormones and other breast cancer biomarkers. The WISER study will contribute to a better understanding of the effect of aerobic exercise on the hormonal and physiological profiles of healthy, sedentary, young women and help determine the efficacy of such an intervention on breast cancer risk prevention.

APPENDIX 2

Poster abstract for International Society for Behavioral Nutrition and Physical Activity, June 17-20, 2009 in Lisbon, Portugal

Title: Is Body Mass Index (BMI) A Good Indicator of Health and Healthy Behavior in Young, Sedentary Women?

Alma J. Smith,[1] Beth Kaufman,[1] Andrea Arikawa,[1] Maureen O'Dougherty,[1] Holly Jakits,[1] Kathryn H. Schmitz[2] Mindy Kurzer[1] [1]*University of Minnesota, Food Science and Nutrition,* [2] *University of Pennsylvania, Department of Biostatistics & Epidemiology*

Purpose: Body mass index (BMI) is extensively used as a convenient and fairly accurate surrogate measure of body composition and health. Its use as an indicator of health behavior, however, has not been thoroughly explored in young, sedentary women. The objective of this study was to examine whether BMI is associated with measures of health and healthy behaviors in this population.

Methods: Baseline data was collected from 246 premenopausal women enrolled in an exercise intervention on bone mineral content and percent body fat from dual energy x-ray absorptiometry (DXA). Three-day food records were used to assess intake of dietary fiber, calcium, saturated fat and trans fat. Total physical activity and aerobic fitness were measured by the modifiable physical activity questionnaire and a sub-maximal treadmill fitness test,

respectively. Women were divided into healthy and overweight BMI (• 25) categories. Associations were adjusted for age, race and education level.

Findings: BMI was significantly associated with increasing percent body fat and bone mineral content. BMI was inversely associated with measured aerobic fitness but not with self-reported physical activity. The only eating behavior to be significantly associated with increasing BMI levels was decreased dietary fiber intake.

Conclusions: In this study, BMI was significantly associated not only with body composition measures but also aerobic fitness and fiber intake. Differences in self-reported physical activity, however, were not significantly explained by differences in BMI. Therefore, BMI seems to be a good indicator of objective measures of fitness and healthy eating behaviors in young, sedentary women.

APPENDIX 3

Poster abstract for Era of Hope meeting, August 2-5, 2011 in Orlando, FL.

Title: Effects of Aerobic Exercise on Premenopausal Sex Hormone Levels, Estrogen Metabolism, and Menstrual Cycle Characteristics: Results of the WISER Study, A Randomized Clinical Trial in Healthy, Sedentary, Eumenorrheic Women.

Background: Physical inactivity is a modifiable risk behavior for breast cancer. Although the manner by which exercise might decrease breast cancer risk is multifaceted and not well understood, several hormonal mechanisms consistent with decreased risk have been proposed. These include favorable alterations in sex hormone and sex hormone binding globulin (SHBG) levels, estrogen metabolism, and menstrual cycle characteristics. The Women in Steady Exercise Research (WISER) study was a large, randomized clinical trial investigating the effects of aerobic exercise on such changes in healthy, young premenopausal women.

Methods: 391 sedentary, healthy, eumenorrheic women, 18-35 years of age, were randomized into an exercise intervention of 30 minutes of aerobic exercise 5 times a week for approximately 16 weeks (n = 212), or into a usual-lifestyle control group (n = 179). Women who completed the study (n=319) provided fasting blood samples and 72-hour urine collections at baseline and at the end of the 16-week period. Radioimmunoassay kits were used to measure serum levels of estradiol, estrone sulfate, testosterone, and SHBG in the midfollicular phase and progesterone in the midluteal phase. Liquid chromatography-mass spectrometry methods were used to measure urinary midfollicular estrone (E_1) and estradiol (E_2) and their metabolites 2-

hydroxyestrone (2-OHE_1), 2-hydroxyestradiol (2-OHE_2), 4-hydroxyestrone (4-OHE_1), 4-hydroxyestradiol (4-OHE_2), 16-hydroxyestrone (16-OHE_1), estriol (E_3), 2-methoxyestrone (2-$MeOE_1$), 2-methoxyestradiol (2-$MeOE_2$), 4-methoxyestrone (4-$MeOE_1$), and 4-methoxyestradiol (4-$MeOE_2$). Commercial ovulation kits and self-reported, monthly menstrual logs were used to determine timing and occurrence of ovulation and changes in follicular, luteal, and menstrual cycle lengths.

Results: Compared to the controls (n = 153), exercisers (n = 166) experienced a significant decrease in body fat and significant increases in aerobic fitness and lean body mass. By design, body weight remained unchanged in either group. No significant changes were found between groups for any of the serum endpoints; the exercise group had a significant within-group decrease in progesterone. Ratio of urinary 2-OHE_1 to 16-OHE_1 levels increased significantly in the exercise group and this increase differed significantly from the change in the control group. No other differences were found in urinary endpoints. No significant differences in menstrual cycle characteristics were found between or within groups.

Conclusions: In premenopausal women, 16 weeks of 150 minutes per week of moderate aerobic exercise resulted in favorable changes in estrogen metabolism, namely an increased 2-to16 OHE_1 ratio, but no changes in endogenous sex hormone and SHBG levels or menstrual cycle characteristics. Results from this randomized clinical study support the hypothesis that changes in estrogen metabolism may help mediate the beneficial effects of exercise on breast cancer.

APPENDIX 4

Manuscript published by CEBP, July 2011. Cancer Epidemiol Biomarkers Prev 2011;20:1098-1106).

Effects of Aerobic Exercise on Premenopausal Sex Hormone Levels: Results of the WISER Study, A Randomized Clinical Trial in Healthy, Sedentary, Eumenorrheic Women.

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Running Title: Exercise Effects on Premenopausal Sex Hormones

Key words: Aerobic Exercise, Sex Hormones, Premenopausal Women, Randomized Clinical Trial, Breast Cancer Risk

The project was funded by the National Institutes of Health/National Cancer Institute grant 1U54CA116849-010003, the Department of Defense/ U.S. Army Medical Research and Materiel Command Congressionally Directed Medical Research Programs award #W81XWH-08-1-0301, and the National Institutes of Health/National Center for Research Resources grant M01-RR00400.

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Disclosure Summary: The authors have nothing to disclose.

Clinical Trial Registration Number: NCT00393172

The authors wish to acknowledge the WISER administrative and laboratory staff, the General Clinical Research Center at the University of Minnesota, the Minneapolis YWCAs, and the study participants.

ABSTRACT

Background. It is hypothesized that exercise can lead to a decrease in breast cancer risk through several hormonal and non-hormonal mechanisms. The Women in Steady Exercise Research (WISER) study investigated the effects of aerobic exercise on premenopausal sex hormone levels.

Methods. 391 sedentary, healthy, young eumenorrheic women were randomized into an exercise intervention of 30 minutes of aerobic exercise 5 times a week for approximately 16 weeks (n=212), or into a control group (n=179). Serum levels of estradiol, estrone sulfate, testosterone, and sex hormone binding globulin (SHBG), all in the midfollicular phase, and of progesterone, in the midluteal phase, were measured at baseline and at the end of the 16-week period.

Results. Compared to the controls (n=153), exercisers (n=166) experienced significant increases in aerobic fitness, lean body mass, and decreases in percent body fat. There were no significant changes in body weight and menstrual

cycle length between or within groups. Progesterone decreased significantly in exercisers; however, this reduction was similar to that of the control group. No significant changes between or within groups were found for any of the other sex hormones or SHBG.

Conclusions. In premenopausal women, 16 weeks of 150 minutes per week of moderate aerobic exercise in young women did not significantly alter sex hormone or SHBG levels.

Impact. Any favorable effects that moderate aerobic exercise without an associated weight change may have on breast cancer risk in premenopausal women are unlikely to be a consequence of changes in levels of sex hormones or SHBG.

INTRODUCTION

Despite steady decreases in breast cancer mortality rates, breast cancer continues to be the most frequently diagnosed non-skin cancer and second leading cause of cancer death among women (1). Well-established risk factors for breast cancer include early age at menarche, late age at menopause and first childbirth, nulliparity, family history of breast cancer, benign breast disease, and non-reproductive factors such as hormone-replacement therapy use and physical inactivity (2, 3). Collectively, these factors increase the lifetime exposure of breast tissue to circulating sex hormones, which have been implicated, both experimentally and observationally, in the etiology of breast cancer.

In cultured mammary cancer cells, estrogen has been shown to promote cell proliferation (4, 5). Furthermore, although the role of progesterone in breast cancer is unclear, there is evidence progesterone can potentiate the mitogenic effect of estradiol (6). Although not a steroid hormone, the glycoprotein sex hormone binding globulin (SHBG) is also thought to play a role in breast carcinogenesis not only by regulating the bioavailability of estradiol and testosterone in circulation (7), but also by inhibiting estradiol-mediated cell growth and anti-apoptosis in estrogen-dependent breast cancer cells (8).

In observational studies, elevated levels of circulating sex hormones are strongly associated with decreased risk for breast cancer. In a re-analysis of 13 prospective studies, postmenopausal women with the highest levels of estradiol, estrone, and testosterone had a two-fold increase in breast cancer risk, and those with the highest SHBG

levels had a 34% decreased risk (9). Similarly, studies in premenopausal women have shown associations between increases in breast cancer risk with higher levels of estrogens and androgens and lower levels of progesterone and SHBG (10-21). Importantly, premenopausal levels of estradiol have been associated with postmenopausal breast cancer (16), suggesting exposures during this period may very well play a role in the initiation and promotion of breast cancer.

Physical activity is a modifiable lifestyle that has been associated with reductions in breast cancer risk of approximately 25-30% (22). Although many mechanisms have been suggested for the protective effect of exercise on breast cancer, reduction of circulating levels of sex steroid hormones is one that has been widely suggested (23). This has not been substantially studied in clinical trials, although in a recent, small clinical study of sedentary premenopausal women reported by Williams *et al.* (24), a four-cycle intervention consisting of moderate-intensity aerobic exercise in combination with a caloric restrictive diet resulted in significant decreases in serum estradiol and urinary estrone-1-glucuronide (E1G) and pregnanediol glucoronide (PdG) levels. In contrast, our study, the Women In Steady Exercise Research (WISER) study, was a randomized trial of premenopausal women that investigated the effects of a moderate-to-vigorous exercise intervention independent of diet restriction and weight loss. We specifically sought to determine if the exercise intervention would lead to alterations in levels of sex hormones and SHBG that would be consistent with a decreased risk of breast cancer.

MATERIALS AND METHODS

The WISER study was a randomized, controlled, parallel-arm study that investigated the effects of a 16-week, moderate-to-vigorous intensity, aerobic exercise intervention on breast cancer biomarkers in young, healthy, sedentary, eumenorrheic women. The study was approved by the Human Subjects Review Committee at the University of Minnesota (Institutional Review Board; IRB ID#0505M69867). Written informed consent was obtained from each participant prior to participation. Details of the study design and methods have been described previously (25). Briefly, 391 non-smoking women aged 18-30 years residing in the Minneapolis-St. Paul metropolitan area with a body mass index (BMI) of 18-40 kg/m^2 (inclusive), having a self-reported menstrual cycle length of 24 to 35 days and a sedentary lifestyle (two or less weekly sessions of moderate intensity exercise) were randomized into the WISER study. Exclusion criteria included use of hormonal contraceptives in the past three

months of any form or depot-medroxyprogesterone acetate in the past 12 months, gynecological problems, metabolic or endocrine-related diseases, non-melanoma cancer in the past 5 years, alcohol consumption of > 7 servings per week, current or recent (past 6 months) pregnancy, and body weight changes greater than 10% over the past year. A total of 391 women started the study by completing baseline measurements during the luteal phase of menstrual cycle 1 and the follicular phase of cycle 2.

Randomization to either an exercise intervention or a no-exercise control group occurred after both baseline measurements were taken. Women with menstrual cycles averaging 25-31 days concluded the study with follow-up measurements during the luteal phase of cycle 5 and the follicular phase of cycle 6. Women with menstrual cycle lengths outside this range had follow-up measurements scheduled such that study duration after randomization was no less than 14 weeks and no more than 18 weeks. Specifically, women with menstrual cycle lengths of less than 25 days provided follow-up measurements during the luteal phase of menstrual cycle 6 and the follicular phase of cycle 7, while women with menstrual cycle lengths of more than 31 days completed these measurements during the follicular and luteal phases of cycle 5. Randomization was stratified on baseline BMI tertiles (≤ 22.8, 22.8-26.3, ≥ 26.3) based on the 50^{th} and 75^{th} percentiles from NHANES I data and age (18-24 *vs.* 25-30). Initially, the randomization ratio (exercise:control) was 1:1 but due to the higher dropout rate in the treatment group it was later changed to 60:40 to ensure adequate sample size in both groups was achieved within the projected study timeline. While failure to return for follow-up measures resulted in being dropped out of the study, exercisers were additionally subject to study exclusion if they missed 15 or more exercise sessions. Figure 1 shows the screening, randomization, retention, and completion of WISER participants.

Exercise Intervention

Women randomized to the exercise intervention trained aerobically five times a week for 30 minutes on a treadmill, stair-stepper, or elliptical machine, at a specified intensity based on age-predicted maximal heart rate (max HR) for 4 menstrual cycles (14-18 weeks). All training sessions took place at the University of Minnesota's Recreation Center. However, under special circumstances (housing relocation, time constrains, or traveling issues) participants were allowed to work out at another exercise facility. The exercise intensity was initially set at 65%-70% of the age-predicted max HR and was gradually increased by 5% every four weeks until 80%-85% of age-predicted max HR was reached (stage 1 = 65-70%; stage 2 = 70-75%; stage 3 = 75-80%; stage 4 = 80-85%). A certified personal

trainer provided instruction on how to properly use the exercise machines and thoroughly complete an exercise log after each workout. Trainers supervised exercise sessions and reviewed the exercise logs at least once weekly to monitor adherence and safety. When not meeting with a trainer, participants were expected to complete the remaining of the workout sessions on their own at the specified training facility. Exercise adherence was monitored by a heart rate monitor (Polar Electro Inc., Lake Success, NY) and exercise logs. When the trainer detected a missed exercise session in the exercise logs, she contacted the participant to determine the reason for the missed session and to encourage compliance with the study protocol. Any physical activity performed after randomization and outside the prescribed exercise intervention was assessed with a physical activity questionnaire by a research member at the end of the study.

All participants, regardless of randomization outcome, were advised to maintain their baseline body weight. Control participants were asked to maintain their usual level of physical activity and to not change their eating habits. A thorough description of the exercise intervention has been described previously (25).

Outcome Measures

All biological, anthropometric, and body composition measures were taken at the General Clinical Research Center at the University of Minnesota. Body weight was measured four times during the study (baseline, cycle 3, cycle 4, and at follow up) to the nearest 0.1 kg, using an electronic scale (Scale Tronix, White Plains, NY). Height was measured by a stadiometer at baseline without shoes to the nearest 0.1 cm (Scale Tronix, White Plains, NY). Body mass index was calculated by dividing weight in kg by height in meters squared (kg/m^2). Body composition was assessed at baseline and follow-up luteal phase clinic visits by dual energy x-ray absorptionetry (DXA) using a Lunar Prodigy DXA apparatus (Lunar Radiation Corp., Madison, WI).

A sub-maximal treadmill test was used to assess aerobic fitness at baseline and immediately after the intervention. This workload was then converted to metabolic equivalents (METs) by using a standard conversion formula (26). Details of the fitness protocol have been described previously (25). Self-reported physical activity performed a year prior to the study and during the 4-month follow-up period was assessed by research staff via a modified version of the Modifiable Activity Questionnaire (27). This information was transformed into MET-hours per week (MET-hrs/wk) using commonly accepted MET values (28). Dietary intake was assessed through self-

reported, 3-day food records at baseline and follow-up. Nutrient intake was determined using The Food Processor SQL® by ESHA Research (Salem, OR).

Timing and occurrence of ovulation was assessed using a commercial 9-day Assure LH™ ovulation kit (Conception Technologies, San Diego, CA). This kit assesses for luteinizing hormone (LH) surge via ELISA, with a 96% accuracy rate with home use. For purposes of the study, day of ovulation was considered to be the day after a positive LH surge result. Participants were asked to inform research staff of positive LH surge results each month either by email or phone.

Hormone and SHBG Analysis

Blood samples were drawn between 6:45 and 11:00 am after an overnight fast, centrifuged for 15 minutes (4°C at 1000 x g), serum was separated, aliquoted, and stored frozen at -70°C. Baseline and follow-up blood draws took place during specific days of the menstrual cycles. Midluteal phase blood draws were scheduled 6-9 days after ovulation for analysis of progesterone during cycles 1 and 5 (cycle 6 for women with menstrual cycle lengths of less than 25 days) while midfollicular phase blood draws were on cycle days 7-10 for the analysis of all other sex hormones and SHBG during cycles 2 and 6 (cycle 5 and cycle 7 for women with menstrual cycle lengths of less than 25 days and more than 31 days, respectively).

Serum concentrations of estradiol, estrone sulfate, testosterone, progesterone, and SHBG were measured by laboratory personnel blinded to the intervention status. Commercially available RIA kits (Diagnostic System La2oratories, Webster, TX) were used to measure estradiol (DSL-4400), estrone sulfate (DSL-5400), testosterone (DSL-4100), and progesterone (DSL-3900). An ELISA method (Immuno-Biological Laboratories-America, Minneapolis, MN) was used to measure SHBG (IBL-59106). Free and bioavailable fractions of estradiol and testosterone were calculated using the equations by Vermeulen *et al.* (29) and association constants estimated by Mazer (30). Although not a sex hormone, SHBG will be referred as such in the remainder of the text for ease of expression.

Samples were assayed in duplicate and in batches such that each batch contained both baseline and follow-up samples from each participant and an equal number of exercise and control participants. Two quality control blood samples were included in each batch. The mean intra-assay and inter-assay CVs were 5.7% and 16.6% for

estradiol; 3.7% and 12.7% for estrone sulfate; 6.1% and 23.2% for testosterone; 8.6% and 11.7% for progesterone; and 4.9% and 5.2% for SHBG.

Statistical Analysis

Unadjusted comparisons of baseline characteristics were performed by using Student's *t*-tests for continuous variables and chi-square tests for categorical variables. Baseline associations between sex hormones and measures of body composition, adiposity, fitness, reproductive characteristics, and diet were determined using Spearman correlation coefficients. The main trial analysis assessed the intervention effect on hormones on an intent-to-treat basis such that all samples from participants who completed at least one follow-up blood measure were included in the analysis regardless of compliance level. Comparison of sex hormone levels at baseline, follow-up, and changes from baseline were adjusted for age and BMI strata with a general linear model. Baseline and follow-up analyses were conducted using log transformed hormone values while changes from baseline were analyzed on the original scale. Linear models were calculated using SAS software, version 9.2 (SAS institute Inc, 2008, Cary, NC). $P < 0.05$ was considered statistically significant.

RESULTS

Study participants

As shown in Figure 1, of the 212 and 179 women randomized into the exercise and control groups, 166 (78.3%) and 153 (85.5%), respectively, completed the WISER study ($P = 0.68$). With the exception of education level ($P = 0.10$), women who dropped out of the study were no different than women who completed the study in terms of age, height, weight, BMI, race, ethnicity, marital status, previous contraceptive use, and parity (data not shown). Both baseline and follow-up midfollicular and midluteal phase samples were obtained from 319 and 311 women, respectively. Most of the women who completed the study were single (82%), Caucasian (72%), educated (67% were at college level or higher), and had a normal BMI (63.5%). There were no significant differences in baseline demographic characteristics between the study groups (Table 1).

Baseline associations

At baseline, estradiol was significantly associated with assessed fitness ($r = -0.14$, $P = 0.01$). Testosterone was significantly correlated with age ($r = -0.15$, $P = 0.008$), BMI ($r = 0.13$, $P = 0.02$), and percent body fat ($r = 0.16$, $P =$

0.005). Progesterone was significantly associated with age (r = 0.12, *P* = 0.03). SHBG was positively associated with age (r = 0.11, *P* = 0.05) and negatively associated with BMI (r = -0.22, *P* < 0.001) and percent body fat (r = -0.21, *P* = 0.001). There were no significant associations between any of the sex hormones and self-reported physical activity, energy or alcohol intake, or reproductive factors such as age at menarche.

Treatment adherence

Adherence to the exercise intervention in the WISER study was excellent; on average, exercise participants completed 134 minutes per week of the assigned 150 minutes exercise intervention. Exercise adherence in stage 1 was 97.6% and 85.3% in stage 4. More details about the exercise adherence can be found somewhere else (31) .

Treatment effects

The exercise intervention resulted in a significant increase in aerobic fitness (increase of 0.90 METs reached at 85% of max HR for exercisers *vs.* 0.12 METs for controls) and improvements in body composition measures. Exercisers gained more lean mass (0.55 kg *vs.* 0.07 kg) and lost significantly more fat mass (0.57 kg *vs.* 0.04 kg) and body fat (0.95% *vs.* 0.09%) than controls. No changes in body weight were observed in either group (Table 2).

There were no differences between exercise and control groups in both baseline and follow-up sex hormone or SHBG levels, except that exercisers had significantly lower estrone sulfate, with and without adjustment for baseline levels (Table 3). Similarly, adjustment for baseline levels to changes from baseline comparisons in estrone sulfate resulted in similar means and *P* values as those obtained without the adjustment. Therefore, the results reported in Table 3 for follow-up and changes from baseline in estrone sulfate are age- and BMI-adjusted only. With the exception of progesterone, there were no differences within-group in sex hormone or SHBG levels. Progesterone levels decreased modestly but significantly (*P* = 0.02) in exercisers; however, this reduction was statistically similar to that experienced by control participants. No differences were found between groups in change from baseline in any of the sex hormones or SHBG. Results were consistent when comparisons were restricted to normal weight, overweight, and obese subgroups. There were no significant changes from baseline in menstrual cycle length between or within groups (data not shown).

DISCUSSION

According to the American College of Sports Medicine and the American Heart Association, 30 minutes of moderate-intensity aerobic exercise performed five times a week is consistent with the promotion and maintenance of health (32). The WISER study was the first randomized, controlled study designed to test whether a moderate-to-vigorous exercise regimen resulting in no weight loss would result in changes in circulating levels of blood sex hormones and SHBG associated with reduction of breast cancer risk in premenopausal women.

The exercise intervention in the WISER study resulted in favorable changes in aerobic fitness and body composition measures; however, no significant differences were observed between exercisers and control participants in the changes of serum estradiol, estrone sulfate, testosterone, progesterone, or SHBG. Although the study was not specifically powered to assess for hormonal differences between the two groups, the virtually identical results for these parameters make it unlikely that physiologically important differences were present. Most cross-sectional data are consistent with these null results. For example, previous studies have found no significant associations between physical activity and premenopausal levels of estrone sulfate (33), testosterone (34, 35), progesterone (34, 36), and SHBG (34, 36, 37). As for total and free estradiol, only two (36, 38) of six (33-38) and one (34) of two (33, 34) studies, respectively, have found a significant negative association. In contrast to our results, the one study that evaluated the association between physical activity and both estrone and free testosterone did find significant negative associations (34).

More importantly, our results are consistent with three small clinical studies in premenopausal women. In a study by Rogol *et al.* (39), there were no differences in integrated estradiol and progesterone levels in seventeen subjects who completed one year of endurance training compared to six (nonrandomized) controls. In the WISER pilot study, 15 weeks of moderate-to-vigorous intensity aerobic exercise in fifteen sedentary premenopausal women resulted in no significant changes in urinary estradiol or estrone levels despite a significant, albeit small (1.2 kg) loss in weight (40). In the study of Williams *et al.*, sedentary premenopausal women allocated to 120-240 minutes per week of moderate exercise in combination with a caloric restrictive diet (20-35% of baseline energy requirements) experienced significant reductions in body weight (3.7 kg), serum estradiol, and urinary EIG and PdG levels (24). However, the control participants, who followed an intervention comparable to that of the WISER exercisers (36 minutes of moderate exercise twice a week in addition to unrestrictive, eucaloric diet), did not experience significant changes in body weight or sex steroid levels.

There are different reasons why the exercise intervention of the WISER study may have failed to have resulted in detectable changes in sex hormone and SHBG levels. It has been hypothesized that the effects of exercise on reproductive hormones are mediated by changes in body composition (22, 41). While such changes are less important for cycling premenopausal women as compared to postmenopausal women, in whom the primary source of estrogens is peripheral aromatization of androgens in adipose tissue (42, 43), in our study the lack of any such effects may have been because body composition changes were modest. Thus perhaps a longer exercise intervention would have yielded different results. It is also possible that a more intensive sampling and/or sampling closer to the time of ovulation (when estradiol levels are higher) would have improved our ability to detect changes in hormone concentrations.

Alternatively, it is possible exercise exerts an independent effect on hormone exposure by disrupting hypothalamic function resulting in changes in menstrual cycle characteristics such as delayed onset of menarche, irregular or absent menstrual periods, abnormal or loss of luteal function, and longer menstrual cycle length (22, 41). In our study, although we observed no significant within- or between-group changes in menstrual cycle length, it is possible follicular and luteal phase lengths may have changed significantly even when no changes in menstrual cycle length were detected. We are currently investigating whether the WISER exercise intervention resulted in changes in follicular and luteal phase lengths as well as changes in estrogen metabolism. Finally, it is possible exercise may decrease breast cancer risk in premenopausal women through non-hormonal mechanisms such as changes in endogenous oxidative stress, insulin and glucose metabolism, inflammatory marker levels, and immune function (22, 41). We plan to separately report the effects of the exercise intervention on biomarkers for these mechanisms.

The WISER study is the first clinical trial with randomized controls to study the effects of aerobic exercise on serum reproductive hormone levels in premenopausal women. Strengths include a large sample size, carefully timed follicular and luteal blood samples, and excellent protocol adherence. Findings from this study do not support the hypothesis that, at least in the absence of weight change or obvious menstrual cycle disruption, 150 minutes per week of moderate aerobic exercise leads to reductions in sex hormone concentrations and increases in SHBG concentrations in premenopausal women.

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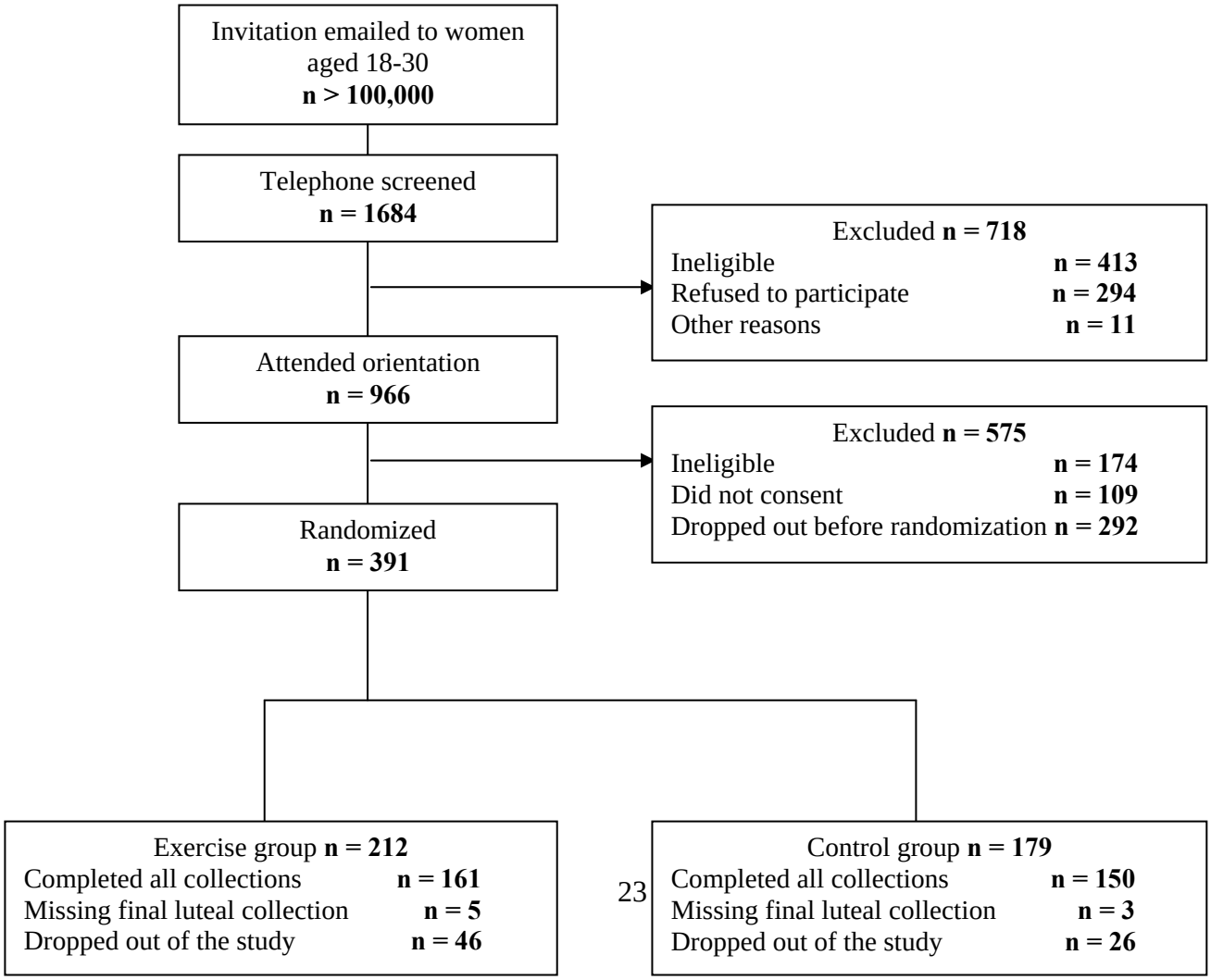

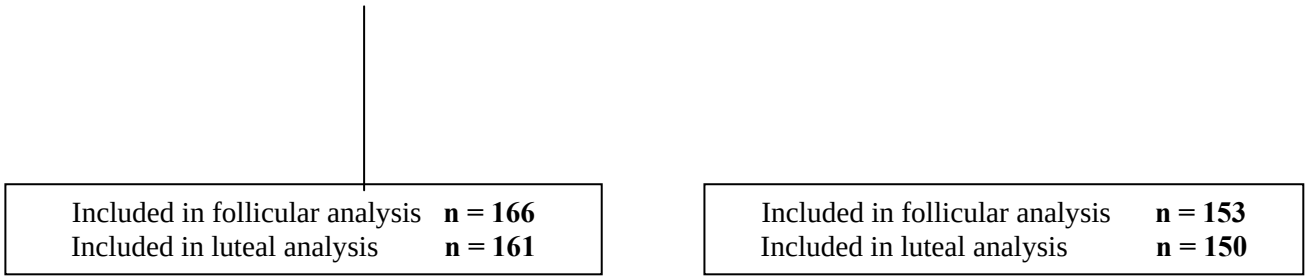

Figure 1. CONSORT diagram showing participant recruitment, screening, randomization, and retention.

Table 1. Baseline Characteristics of Randomized Participants (n = 319) by Treatment Group

	Exercisers n = 166	Controls n = 153	*P*
Age (years)	25.4 ± 3.4	25.2 ± 3.5	0.73
Height (cm)	164.9 ± 6.9	165.4 ± 7.5	0.54
Weight (kg)	67.4 ± 14.6	67.6 ± 14.6	0.94
BMI (kg/m^2)	24.7 ± 4.7	24.7 ± 4.8	0.88
Fat Mass (kg)	24.2 ± 11.2	24.1 ± 10.6	0.90
Percent body fat	36.4 ± 8.8	36.1 ± 8.3	0.77
Lean Mass (kg)	39.7 ± 5.0	40.0 ± 5.2	0.63
Weight Categories			0.38
Underweight (BMI < 18.5)	1 (1%)	1 (1%)	
Normal (18.5 ≤ BMI < 25)	100 (60%)	100 (65%)	
Overweight (25 ≤ BMI < 30)	46 (28%)	30 (20%)	
Obese (BMI > 30)	19 (11%)	22 (14%)	
Race			0.66
White	124 (75%)	107 (70%)	
Black	13 (8%)	12 (8%)	
Asian	20 (12%)	26 (17%)	
Other	9 (5%)	8 (5%)	
Hispanic	8 (5%)	6 (4%)	0.70
Education			0.66
High school or less	11 (7%)	7 (4%)	
Some college	43 (26%)	44 (29%)	
College graduate or more	112 (67%)	102 (67%)	
Marital Status			0.89
Never married or Partnered	138 (83%)	124 (81%)	
Married or Partnered	25 (15%)	26 (17%)	
Separated or Divorced	3 (2%)	3 (2%)	
Age at menarche (years)[a]	12.8 ± 1.5	12.7 ± 1.3	0.53
Nulliparous	154 (93%)	144 (94%)	0.63
Previously using Contraceptives	84 (51%)	82 (54%)	0.49
Family History of Breast Cancer [b]			0.60
No	129 (96%)	114 (97%)	
Yes	5 (4%)	3 (3%)	
Self-reported Diet (kcal/day)[c]	1901 ± 420	1933 ± 525	0.56
Self-reported Physical Activity (MET-hrs/wk)	21.9 ± 16.6	21.8 ± 17.5	0.97
Assessed Fitness (METs at 85% max HR)	6.9 ± 1.5	7.1 ± 1.5	0.45

Note: For continuous variables, values are mean ± SD, and *P*-values are based on Student's *t*-tests. For categorical variables, *P*-values are based on chi-square tests.
[a] n = 310, [b] n = 251, [c] n = 312

Table 2. Changes in Fitness, Body Weight, Body Composition, and Energy Intake

	Baseline	Follow-up	Change from Baseline
METs reached at 85% of max HR	n=319	n=309	n=309
Exercise	7.0 ± 0.1	7.8 ± 0.1	0.90 [a] ± 0.07
Control	7.0 ± 0.1	7.1 ± 0.1	0.12 ± 0.08
	0.59	**< 0.0001**	**< 0.0001**
Weight (kg)	n=319	n=312	n=312
Exercise	68.0 ± 0.7	68.0 ± 0.7	-0.03 ± 0.2
Control	68.8 ± 0.8	69.0 ± 0.8	0.03 ± 0.2
P-value	0.41	0.37	0.83
BMI (kg/m2)	n=319	n=312	n=312
Exercise	25.0 ± 0.2	25.0 ± 0.2	-0.01 ± 0.06
Control	25.2 ± 0.2	25.2 ± 0.2	0.01 ± 0.06
P-value	0.45	0.50	0.85
Fat Mass (kg)	n=319	n=317	n=317
Exercise	24.6 ± 0.5	24.1 ± 0.5	-0.57 [a] ± 0.1
Control	25.0 ± 0.5	25.0 ± 0.5	-0.04 ± 0.2
P-value	0.56	0.17	**0.013**
Percent Body Fat	n=319	n=317	n=317
Exercise	36.9 ± 0.4	35.9 ± 0.4	-0.95 [a] ± 0.2
Control	37.1 ± 0.4	37.0 ± 0.4	-0.09 ± 0.2
P-value	0.70	0.06	**0.0003**
Lean Mass (kg)	n=319	n=317	n=317
Exercise	39.9 ± 0.4	40.4 ± 0.4	0.55 [a] ± 0.1
Control	40.3 ± 0.4	40.4 ± 0.4	0.07 ± 0.1
P-value	0.41	0.93	**0.003**
Self-reported Diet (kcal/day)	n=312	n=303	n=298
Exercise	1898 ± 38	1895 ± 51	-18 ± 51
Control	1932 ± 40	1711 ± 54	224 [a] ± 53
P-value	0.53	**0.011**	**0.004**

Note: Values are means ± SE. Positive values represent increases from baseline while negative values represent decreases from baseline.

[a] Significant within-group change from baseline ($P < 0.05$).

Table 3. Baseline, Follow-up, and Changes from Baseline in Sex Hormone and SHBG Levels

Sex Hormone n = 319	Baseline	Follow-up	Change from Baseline
Estradiol (E_2) (pg/mL)			
Exercisers	56 (50-63)	60 (56-64)	2.3 ± 2.8
Controls	58 (51-65)	62 (58-66)	1.4 ± 3.0
P-value	0.66	0.44	0.83
Bioavailable E_2 (pg/mL)			
Exercisers	39 (36-41)	40 (37-43)	1.4 ± 1.9
Controls	41 (39-44)	43 (40-46)	1.1 ± 2.0
P-value	0.14	0.13	0.89
Free E_2 (pg/mL)			
Exercisers	1.3 (1.2-1.4)	1.4 (1.3-1.4)	0.05 ± 0.06
Controls	1.4 (1.3-1.5)	1.5 (1.4-1.6)	0.04 ± 0.07
P-value	0.14	0.13	0.89
Estrone sulfate (ng/mL)			
Exercisers	2.0 (1.8-2.2)	2.0 (1.9-2.2)	-0.04 ± 0.06
Controls	2.2 (2.0-2.4)	2.3 (2.1-2.4)	-0.01 ± 0.06
P-value	**0.040**	**0.017**	0.74
Testosterone (T) (pg/mL)			
Exercisers	451 (423-482)	446 (419-475)	-8.8 ± 9.0
Controls	473 (442-506)	464 (435-495)	-13.6 ± 9.5
P-value	0.32	0.38	0.71
Bioavailable T (pg/mL)			
Exercisers	204 (187-222)	204 (188-222)	-2.7 ± 5.6
Controls	224 (205-245)	225 (206-246)	-3.0 ± 5.8
P-value	0.13	0.11	0.97
Free T (pg/mL)			
Exercisers	8.8 (7.9-9.7)	8.7 (8.0-9.5)	-0.13 ± 0.25
Controls	9.2 (8.3-10.2)	9.6 (8.8-10.5)	-0.04 ± 0.30
P-value	0.50	0.11	0.80
Progesterone (ng/mL)[a]			
Exercisers	12 (10-14)	10 (8-11)	-2.2[b] ± 0.9
Controls	13 (11-15)	12 (10-14)	-1.1 ± 1.0
P-value	0.61	0.12	0.42
SHBG (nmol/L)			
Exercisers	27 (25-30)	26 (24-29)	-1.3 ± 1.0
Controls	25 (23-27)	24 (22-26)	-1.8 ± 1.1
P-value	0.15	0.08	0.74

NOTE: Values are age- and BMI-adjusted geometric means (95% CI) for baseline and follow-up, and mean ± SE for changes in hormone levels. Positive values represent increases from baseline while negative values represent decreases from baseline.

[a] n = 311

[b] Significant within-group change from baseline ($P < 0.05$)